Shiga toxin producing *Escherichia coli* O174: A persistent STEC serogroup displaying virulence features of Uropathogenic *E. coli*

Rosangela Tozzoli
EU Reference Laboratory for *E. coli*
Istituto Superiore di Sanità

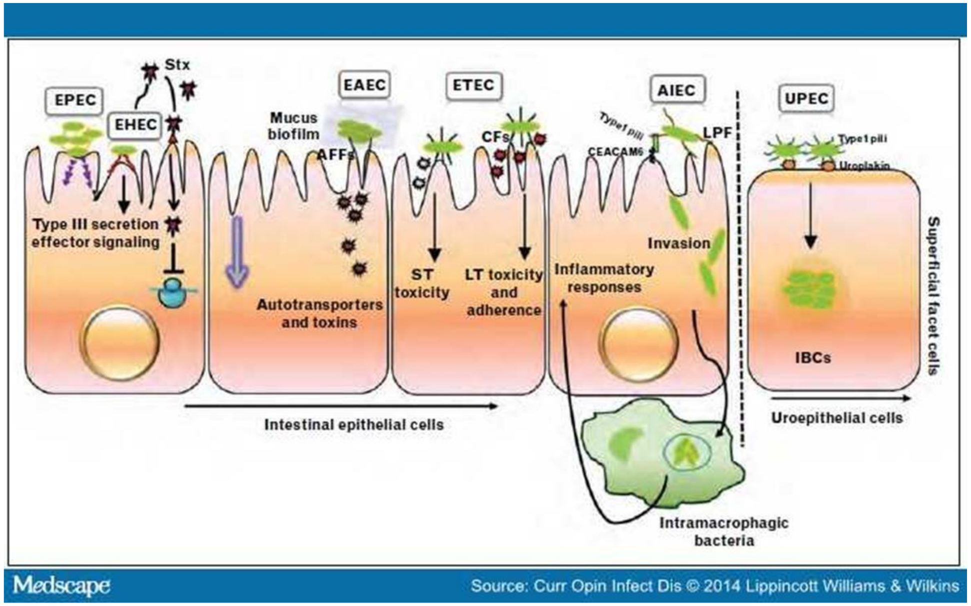
Disease caused by pathogenic *E. coli*

Urinary tract infections

ExPEC

Sepsis/meningitis

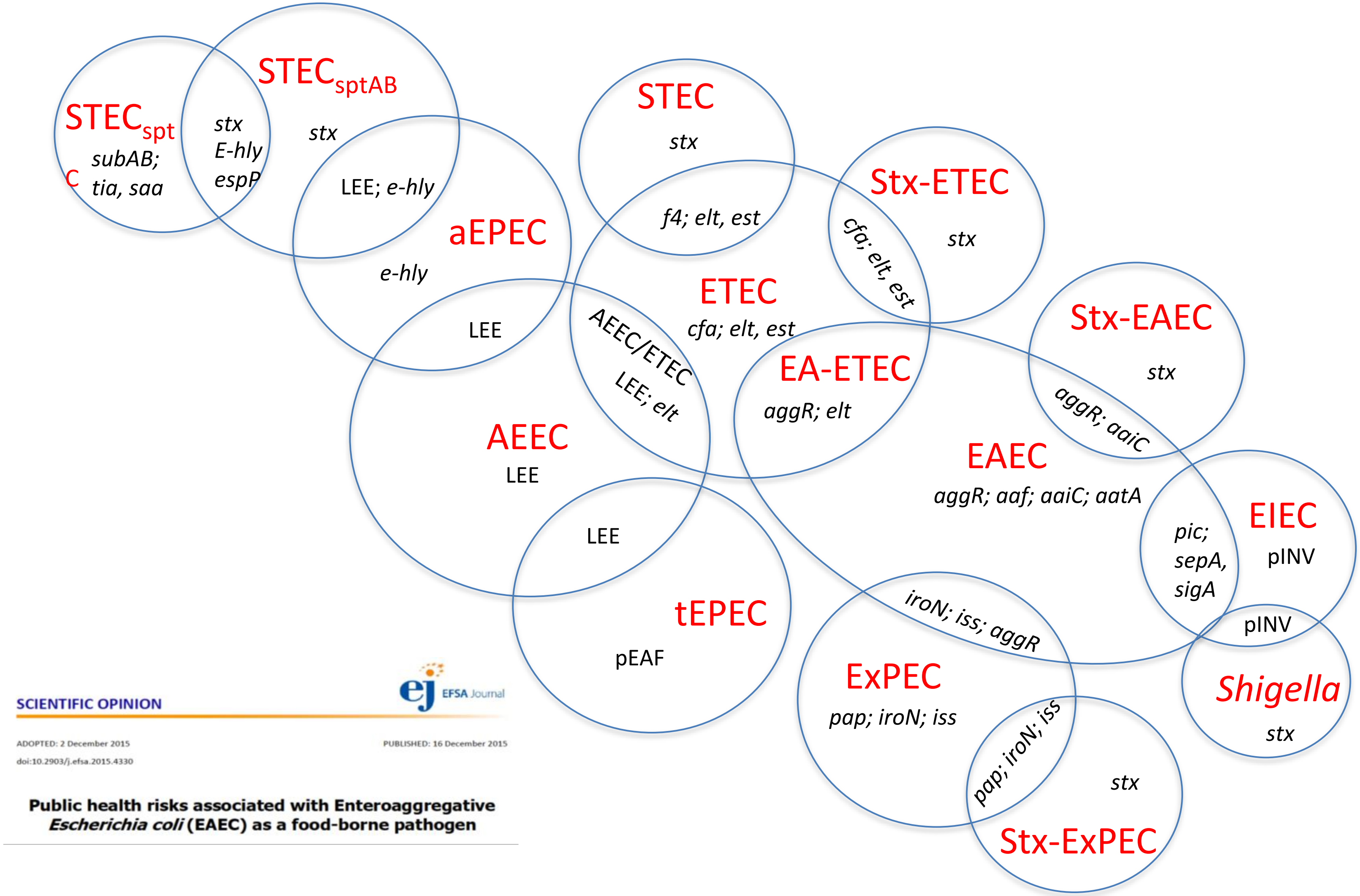
Enteric/diarrhoeal disease **DEC**



Source: Curr Opin Infect Dis © 2014 Lippincott Williams & Wilkins

Different virulence mechanisms, according to their set of virulence determinants

The pathogenic *E. coli* Continuum



$\text{STEC}_{\text{sptC}}$
subAB; tia, saa

stx
E-hly
espP

$\text{STEC}_{\text{sptAB}}$
stx

LEE; *e-hly*

aEPEC
e-hly

LEE

STEC
stx

f4; elt, est

Stx-ETEC
cfa; elt, est
stx

ETEC
cfa; elt, est

AEEC/ETEC
LEE; *elt*

AEEC
LEE

LEE

tEPEC
pEAF

EA-ETEC
aggR; elt

Stx-EAEC
stx
aggR; aaiC

EAEC
aggR; aaf; aaiC; aatA

pic; sepA, sigA

EIEC
pINV

pINV

Shigella
stx

iroN; iss; aggR

ExPEC
pap; iroN; iss

pap; iroN; iss

stx
Stx-ExPEC

SCIENTIFIC OPINION

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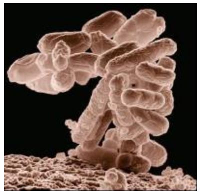
Public health risks associated with Enteroaggregative *Escherichia coli* (EAEC) as a food-borne pathogen

Cross-over pathotype combinations may occur

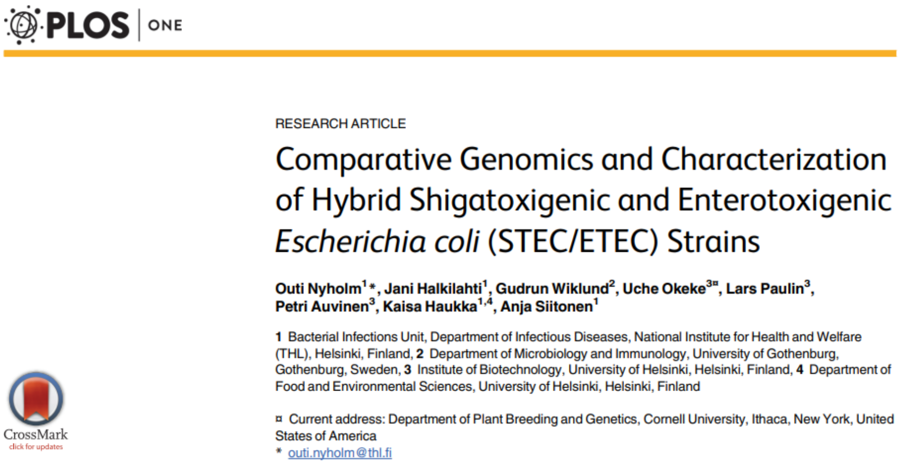
2011 - German outbreak E. coli O104:H4:
4000 human cases 850 HUS 50 deaths



Staked-brick adhesion

Stx-phage

Atypical virulence combination between EAEC and STEC



PLOS | ONE

RESEARCH ARTICLE

Comparative Genomics and Characterization of Hybrid Shigatoxigenic and Enterotoxigenic *Escherichia coli* (STEC/ETEC) Strains

Outi Nyholm[1]*, Jani Halkilahti[1], Gudrun Wiklund[2], Uche Okeke[3¤], Lars Paulin[3], Petri Auvinen[3], Kaisa Haukka[1,4], Anja Siitonen[1]

1 Bacterial Infections Unit, Department of Infectious Diseases, National Institute for Health and Welfare (THL), Helsinki, Finland, **2** Department of Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden, **3** Institute of Biotechnology, University of Helsinki, Helsinki, Finland, **4** Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland

¤ Current address: Department of Plant Breeding and Genetics, Cornell University, Ithaca, New York, United States of America
* outi.nyholm@thl.fi

Hybrid ExPEC-STEC

RESEARCH

Enterohemorrhagic *Escherichia coli* Hybrid Pathotype O80:H2 as a New Therapeutic Challenge

Nurcan Soysal, Patricia Mariani-Kurkdjian, Yasmine Smail, Sandrine Liguori, Malika Gouali, Estelle Loukiadis, Patrick Fach, Mathias Bruyand, Jorge Blanco, Philippe Bidet, Stéphane Bonacorsi

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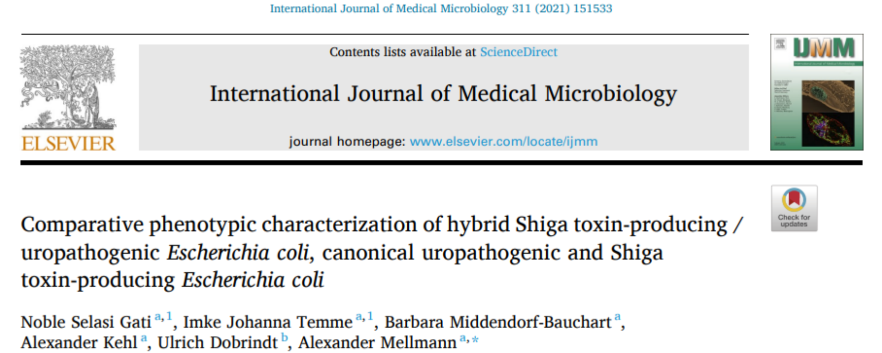
Volume 27, Number 3—March 2021

Research

Genomic Characterization of *hlyF*-positive Shiga Toxin–Producing *Escherichia coli*, Italy and the Netherlands, 2000–2019

Federica Gigliucci✉ , Angela H.A.M. van Hoek, Paola Chiani, Arnold Knijn, Fabio Minelli, Gaia Scavia, Eelco Franz, Stefano Morabito, and Valeria Michelacci
Author affiliations: Istituto Superiore di Sanità, Rome, Italy (F. Gigliucci, P. Chiani, A. Knijn, F. Minelli, G. Scavia, S. Morabito, V. Michelacci);
H.A.M. van Hoek, E. Franz)



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Comparative phenotypic characterization of hybrid Shiga toxin-producing / uropathogenic *Escherichia coli*, canonical uropathogenic and Shiga toxin-producing *Escherichia coli*

Noble Selasi Gati[a,1], Imke Johanna Temme[a,1], Barbara Middendorf-Bauchart[a], Alexander Kehl[a], Ulrich Dobrindt[b], Alexander Mellmann[a,*]

STEC: Zoonotic origin

Natural reservoir

Routes of transmission:

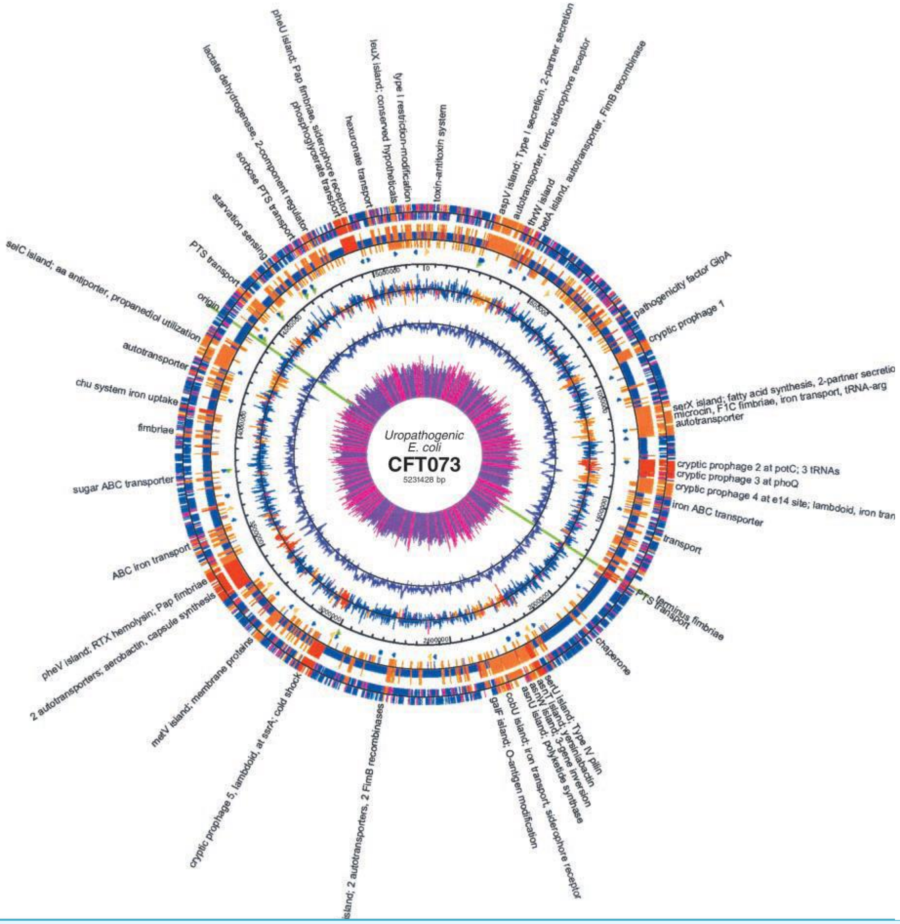
Foodborne - Primary contamination of meat and milk

Environmental contamination

Waterborne: Drinking and recreational water

Direct contact with infected animals

Uropathogenic *E. coli* (UPEC)



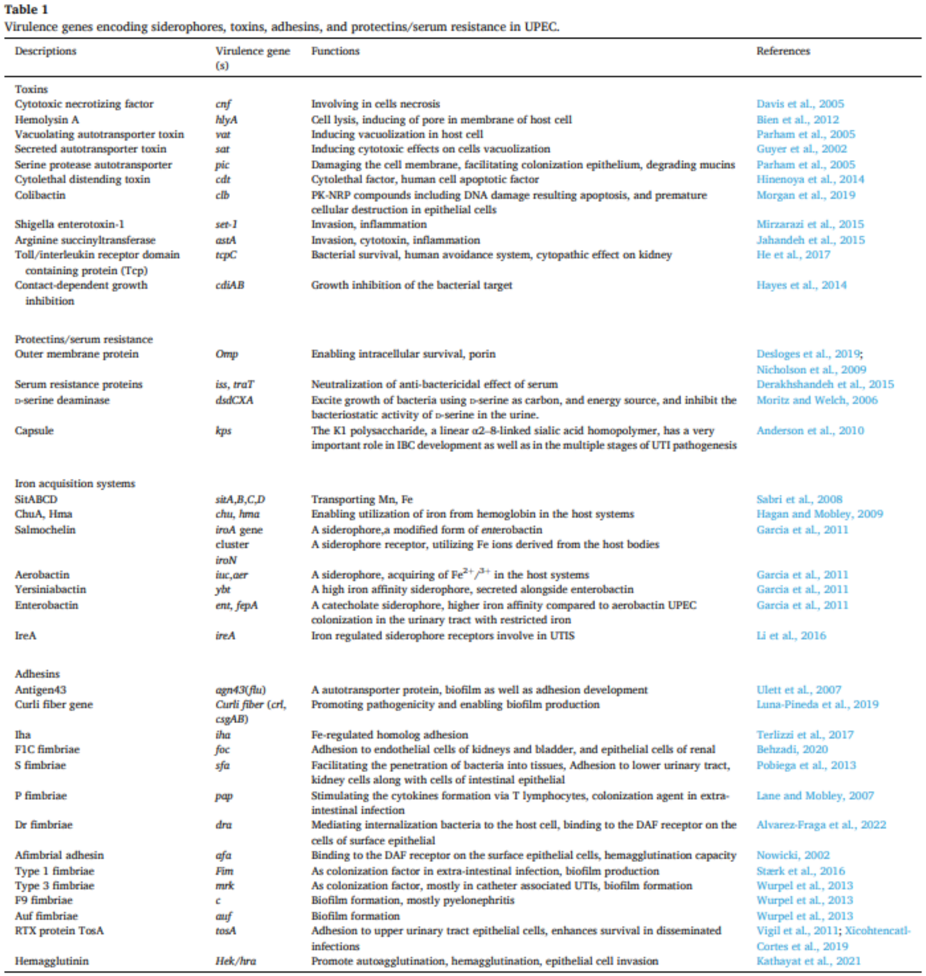


Table 1
Virulence genes encoding siderophores, toxins, adhesins, and protectins/serum resistance in UPEC.

Descriptions	Virulence gene (s)	Functions	References
Toxins			
Cytotoxic necrotizing factor	*cnf*	Involving in cells necrosis	Davis et al., 2005
Hemolysin A	*hlyA*	Cell lysis, inducing of pore in membrane of host cell	Bien et al., 2012
Vacuolating autotransporter toxin	*vat*	Inducing vacuolization in host cell	Parham et al., 2005
Secreted autotransporter toxin	*sat*	Inducing cytotoxic effects on cells vacuolization	Guyer et al., 2002
Serine protease autotransporter	*pic*	Damaging the cell membrane, facilitating colonization epithelium, degrading mucins	Parham et al., 2005
Cytolethal distending toxin	*cdt*	Cytolethal factor, human cell apoptotic factor	Hinenoya et al., 2014
Colibactin	*clb*	PK-NRP compounds including DNA damage resulting apoptosis, and premature cellular destruction in epithelial cells	Morgan et al., 2019
Shigella enterotoxin-1	*set-1*	Invasion, inflammation	Mirzarazi et al., 2015
Arginine succinyltransferase	*astA*	Invasion, cytotoxin, inflammation	Jahandeh et al., 2015
Toll/interleukin receptor domain containing protein (Tcp)	*tcpC*	Bacterial survival, human avoidance system, cytopathic effect on kidney	He et al., 2017
Contact-dependent growth inhibition	*cdiAB*	Growth inhibition of the bacterial target	Hayes et al., 2014
Protectins/serum resistance			
Outer membrane protein	*Omp*	Enabling intracellular survival, porin	Desloges et al., 2019; Nicholson et al., 2009
Serum resistance proteins	*iss, traT*	Neutralization of anti-bactericidal effect of serum	Derakhshandeh et al., 2015
D-serine deaminase	*dsdCXA*	Excite growth of bacteria using D-serine as carbon, and energy source, and inhibit the bacteriostatic activity of D-serine in the urine.	Moritz and Welch, 2006
Capsule	*kps*	The K1 polysaccharide, a linear α2–8-linked sialic acid homopolymer, has a very important role in IBC development as well as in the multiple stages of UTI pathogenesis	Anderson et al., 2010
Iron acquisition systems			
SitABCD	*sitA,B,C,D*	Transporting Mn, Fe	Sabri et al., 2008
ChuA, Hma	*chu, hma*	Enabling utilization of iron from hemoglobin in the host systems	Hagan and Mobley, 2009
Salmochelin	*iroA* gene cluster *iroN*	A siderophore,a modified form of *entero*bactin A siderophore receptor, utilizing Fe ions derived from the host bodies	Garcia et al., 2011
Aerobactin	*iuc,aer*	A siderophore, acquiring of $Fe^{2+}/^{3+}$ in the host systems	Garcia et al., 2011
Yersiniabactin	*ybt*	A high iron affinity siderophore, secreted alongside enterobactin	Garcia et al., 2011
Enterobactin	*ent, fepA*	A catecholate siderophore, higher iron affinity compared to aerobactin UPEC colonization in the urinary tract with restricted iron	Garcia et al., 2011
IreA	*ireA*	Iron regulated siderophore receptors involve in UTIS	Li et al., 2016
Adhesins			
Antigen43	*agn43(flu)*	A autotransporter protein, biofilm as well as adhesion development	Ulett et al., 2007
Curli fiber gene	*Curli fiber (crl, csgAB)*	Promoting pathogenicity and enabling biofilm production	Luna-Pineda et al., 2019
Iha	*iha*	Fe-regulated homolog adhesion	Terlizzi et al., 2017
F1C fimbriae	*foc*	Adhesion to endothelial cells of kidneys and bladder, and epithelial cells of renal	Behzadi, 2020
S fimbriae	*sfa*	Facilitating the penetration of bacteria into tissues, Adhesion to lower urinary tract, kidney cells along with cells of intestinal epithelial	Pobiega et al., 2013
P fimbriae	*pap*	Stimulating the cytokines formation via T lymphocytes, colonization agent in extra-intestinal infection	Lane and Mobley, 2007
Dr fimbriae	*dra*	Mediating internalization bacteria to the host cell, binding to the DAF receptor on the cells of surface epithelial	Alvarez-Fraga et al., 2022
Afimbrial adhesin	*afa*	Binding to the DAF receptor on the surface epithelial cells, hemagglutination capacity	Nowicki, 2002
Type 1 fimbriae	*Fim*	As colonization factor in extra-intestinal infection, biofilm production	Stærk et al., 2016
Type 3 fimbriae	*mrk*	As colonization factor, mostly in catheter associated UTIs, biofilm formation	Wurpel et al., 2013
F9 fimbriae	*c*	Biofilm formation, mostly pyelonephritis	Wurpel et al., 2013
Auf fimbriae	*auf*	Biofilm formation	Wurpel et al., 2013
RTX protein TosA	*tosA*	Adhesion to upper urinary tract epithelial cells, enhances survival in disseminated infections	Vigil et al., 2011; Xicohtencatl-Cortes et al., 2019
Hemagglutinin	*Hek/hra*	Promote autoagglutination, hemagglutination, epithelial cell invasion	Kathayat et al., 2021

The common
source of UPEC is the host's own intestinal flora

From: F. Firoozeh et al, 2022

Genomic Surveillance of STEC in Italy

Isolation of STEC strains at ISS (NRL for *E. coli*) or delivery to ISS of STEC isolates

Sequecing at ISS and upload in the ISS webplatform

OR

Upload of STEC sequence from regional centres

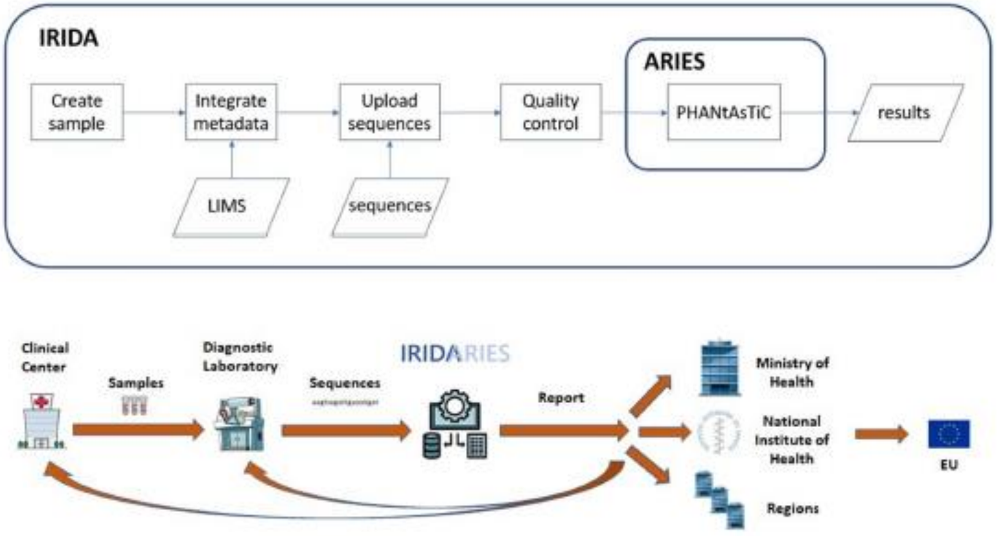
IRIDA

ARIES

STEC strains characterization and comparison with isolates from different sources

Laboratorio Nazionale di Riferimento per *E. coli*
Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria
Reparto di Sicurezza microbiologica degli alimenti e malattie a trasmissione alimentare
Istituto Superiore di Sanità

IRIDA-ARIES system



Knjin A. et al. Frontiers in Public Health, 2023

Phantastic Pipeline:
- ✓ Serotyping
- ✓ MLST-7 genes
- ✓ Virulotyping
- ✓ Stx genes subtyping
- ✓ cgMLST

→

cgMLST analysis among strains of the same serogroup - In case of cluster identification: Immediate report to concerned IRIDA-ARIES users and a report describing the cluster is prepared for further assessment

In February 2024 a genomic cluster of O174 STEC strains was identified

Cluster 394

Consisting in 4 STEC strains *stx1 stx2* from:

- ✓ Bovine faeces (ED1796, ED1798)
- ✓ Cheese samples (ED1802, ED1803)

Isolated from two Vet Institutes in one region in the same period

Looking further in our STEC genomes collection:

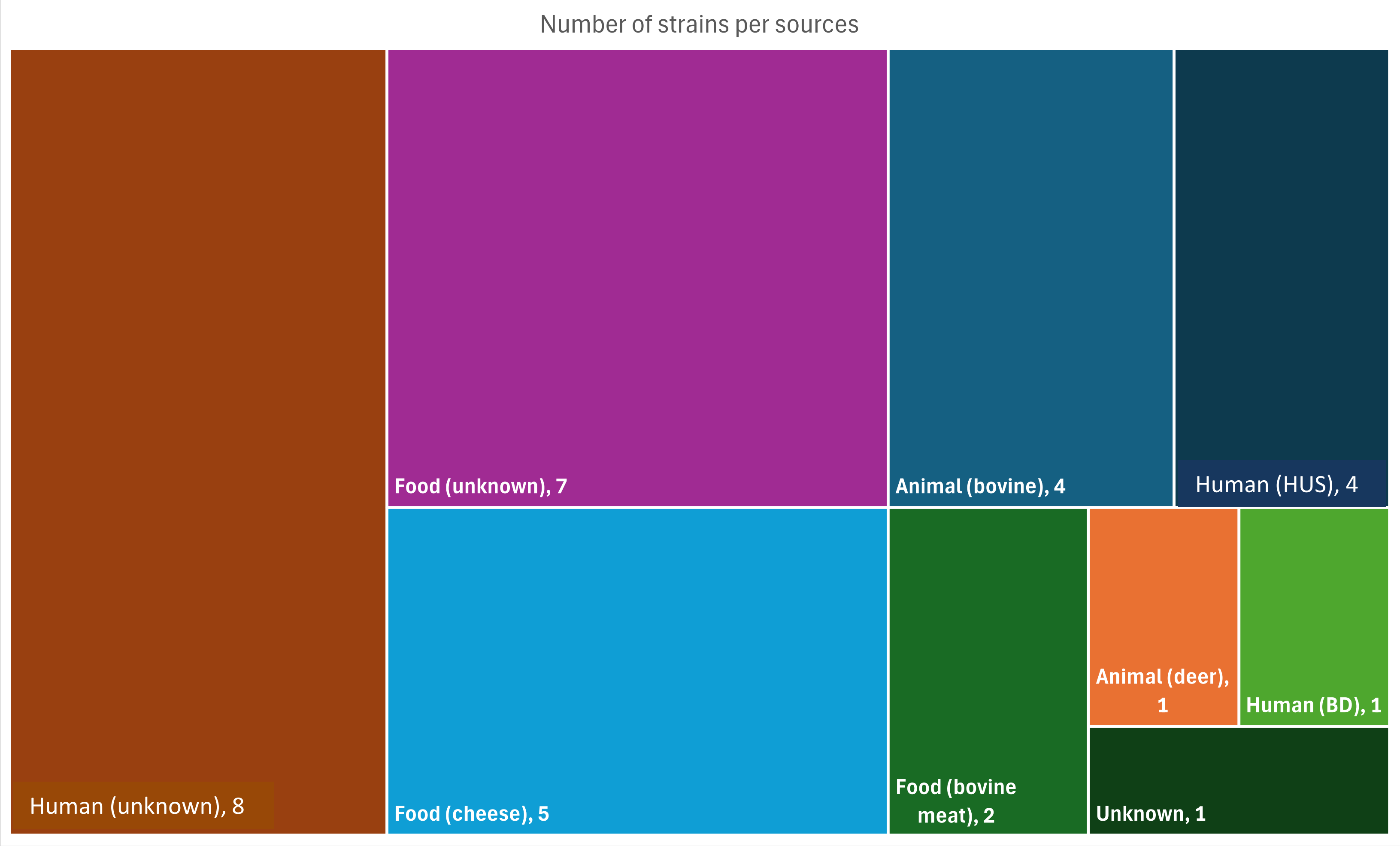
33 STEC strains belonging to O174 serogroup

13 from humans

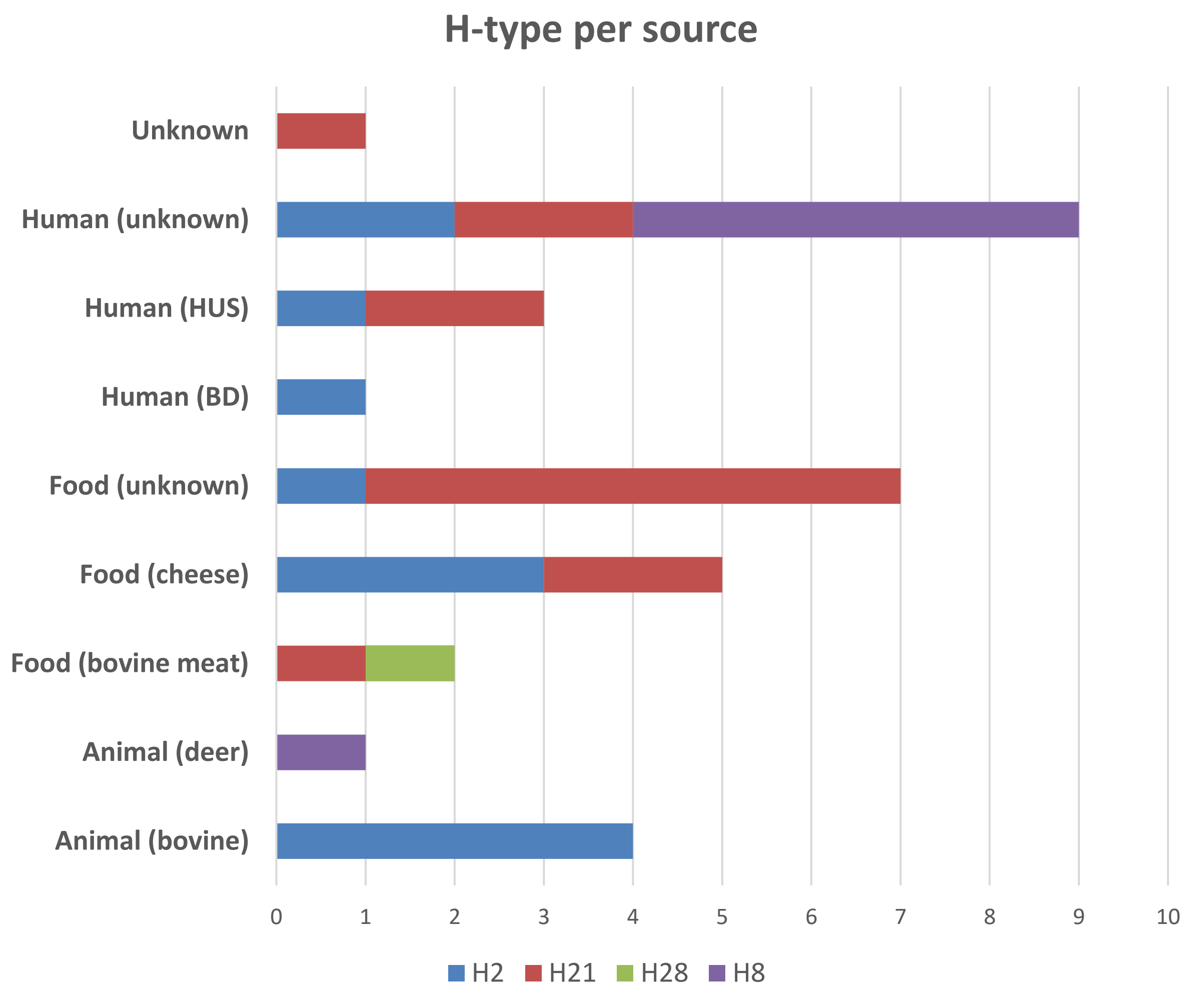
20 from non-human source

Isolated in the time span 1998-2024

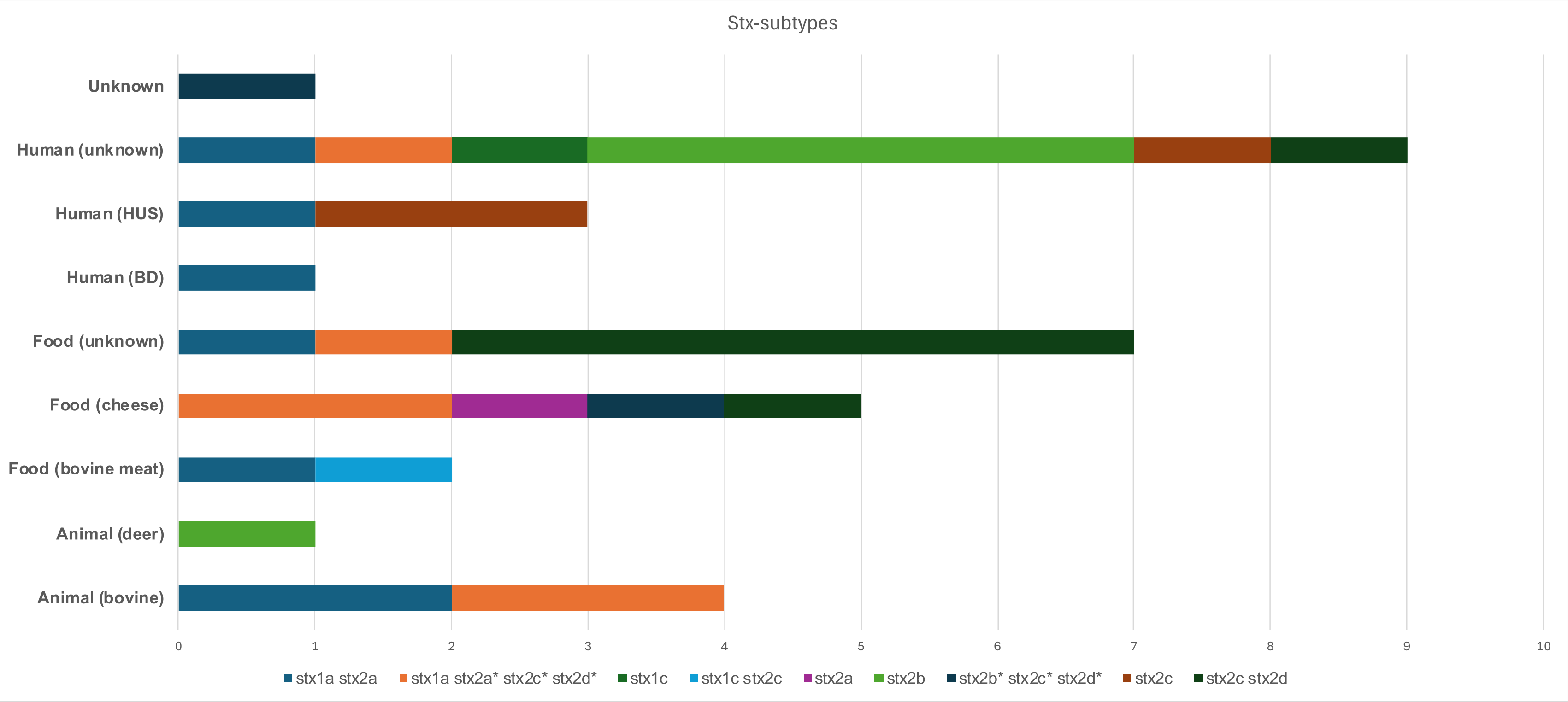
Distribution of O174 strains per source



Distibution of H-types per source



Stx-coding genes subtypes possessed by O174 strains



Strains possessing ExPEC-UPEC virulence genes

cdt: one strain (food)

sen: 5 strains (4 from humans and 1 from deer)

ompT: 16 strains (4 from humans -1 HUS, 7 from food, 5 animals)

AstA: 3 strains (food)

iss: 32 strains (only missing in one strain from bovine meat)

traT: 31 strains (missing in two strains from humans)

iha: 33 strains

papC: 17 strains (2 strains from HUS, 1 from BD, 3 from humans, 3 from cheese, 4 food, 4 from bovine animals)

afa: 1 strain (non human)

hra: 20 strains (1 from HUS, 1 from BD, 3 from humans, 3 from cheese, 5 food-including 1 from bovine meat, 4 from bovine animals, 1 deer)

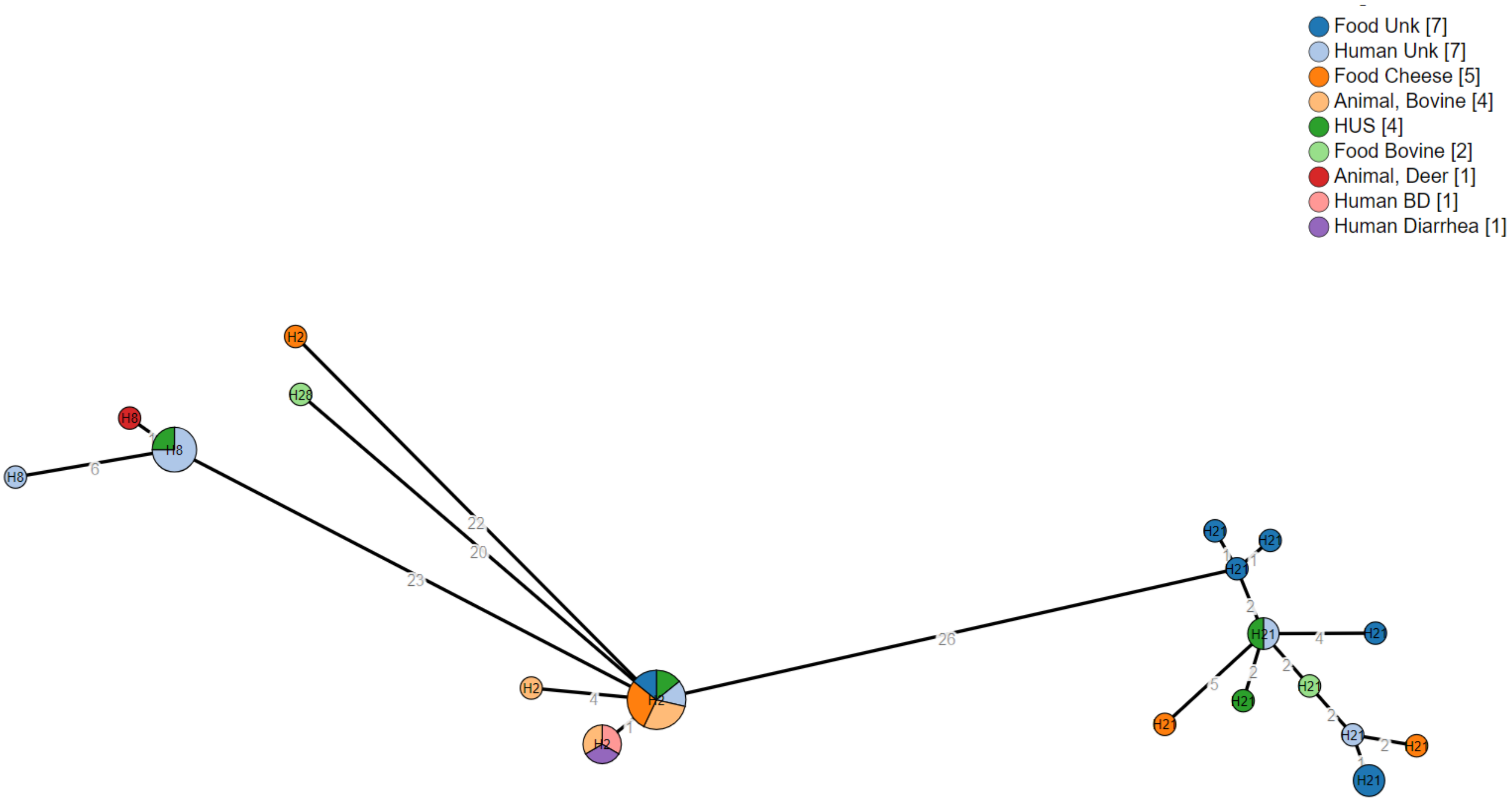
ehxA: 23 strains

subA: 11 strains

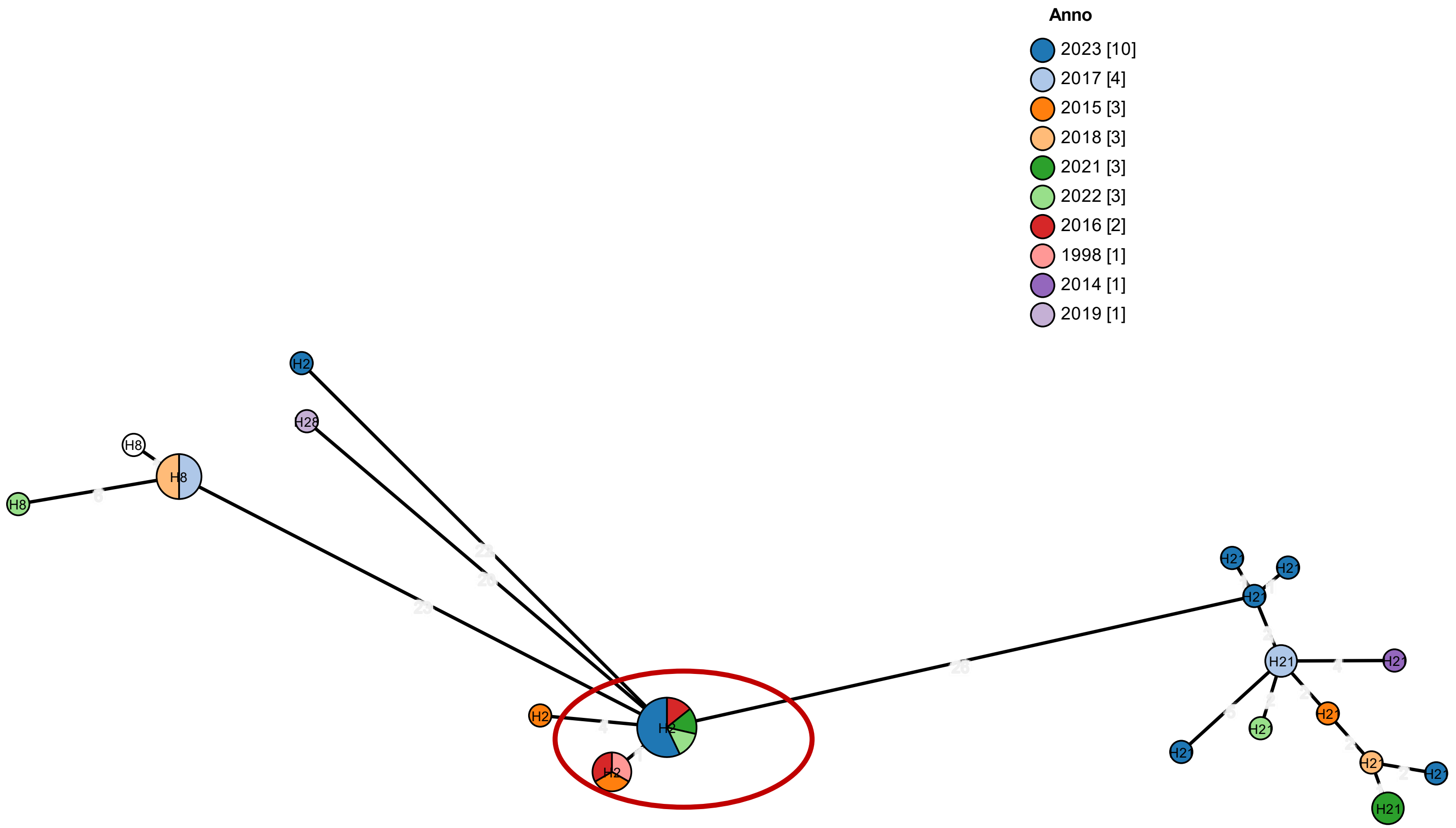
tia: 16 strains

lpfA: 16 strains

cgMLST analysis

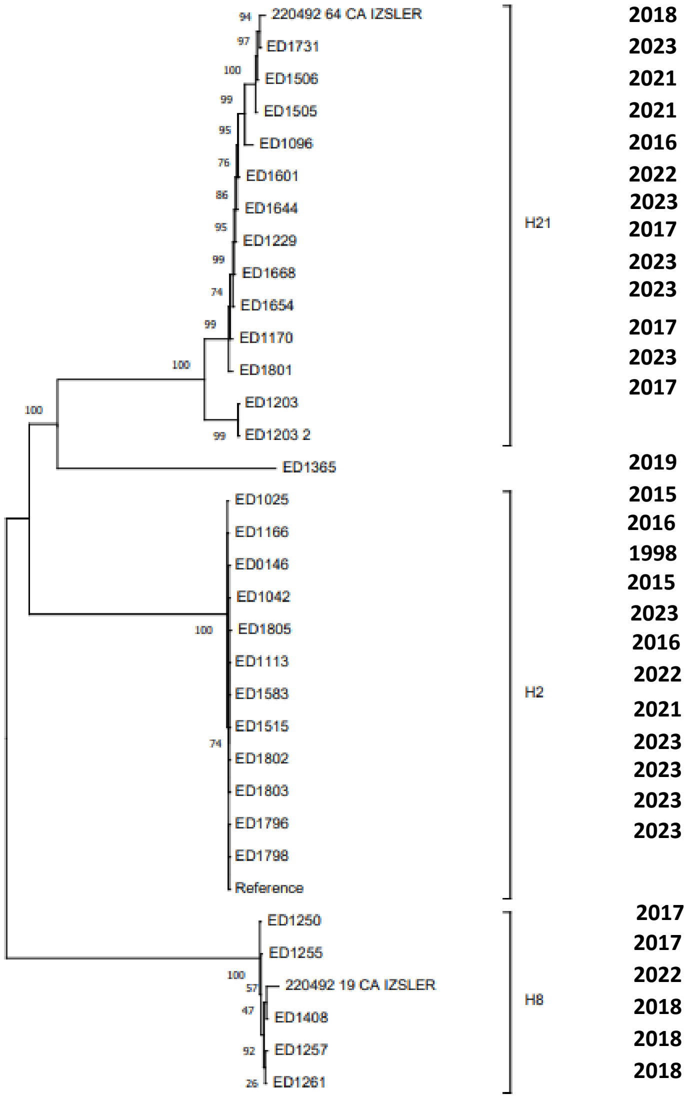


cgMLST analysis



25 years time span

SNPs analysis (core genome)



Concluding remarks

STEC O174 strains isolated in a time-span 30 years in Italy and seem to be persistent (at least H2)

STEC O174 strains isolated from different sources, including severe disease and food

Different *stx* gene subtypes

They possess mosaic virulotypes

Three philogenetically related populations can be identified, each one consisting of strains possessing the same *fliC*

All the three groups contain strains from different origin

⬇

Zoonotic origin

Thank you for your attention!

Sample	Source	Year	O-group	H-type	MLST	Stx-genes subtypes	Virulotype	Cluster ID
ED0146	Animal (bovine)	1998	O174	H2	ST661	*stx1a stx2a*	cba, ehxa, espp, gad, hra, iha, iss, lpfa, ompt, papc, saa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1025	Animal (bovine)	2015	O174	H2	ST661	*stx1a stx2a*	cea, ehxa, espp, gad, hra, iha, iss, lpfa, papc, saa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1042	Human (unknown)	2015	O174	H2	ST661	*stx1a stx2a* stx2c* stx2d**	celb, ehxa, espp, gad, hra, iha, iss, lpfa, papc, saa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1096	Food (unknown)	2016	O174	H21	ST677	*stx1a stx2a* stx2c* stx2d**	asta, gad, hra, iha, iss, lpfa, stx2a, stx2b, terc, trat	
ED1113	Human (HUS)	2016	O174	H2	ST661	*stx1a stx2a*	ehxa, espp, gad, hra, iha, iss, lpfa, papc, saa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1166	Human (BD)	2016	O174	H2	ST661	*stx1a stx2a*	celb, ehxa, espp, gad, hra, iha, iss, lpfa, papc, saa, stx1a, stx1b, stx2b, terc, trat	
ED1170	Human (unknown)	2017	O174	H21	ST677	*stx2c*	espi, gad, iha, iss, lpfa, ompt, papc, stx2b, terc, tia	
ED1203	Food (bovine meat)	2017	O174	H21	ST442	*stx1c stx2c*	gad, hra, iha, irea, lpfa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1229	Human (HUS)	2017	O174	H21	ST677	*stx2c*	cea, ehxa, espi, espp, gad, iha, iss, lpfa, papc, saa, stx2a, stx2b, suba, terc, tia, trat	
ED1250	Human (unknown)	2017	O174	H8	ST13	*stx2b*	cba, cea, cia, cma, ehxa, espi, gad, iha, irea, iss, lpfa, senb, stx2a, stx2b, suba, terc, tia, trat	173
ED1255	Human (unknown)	2017	O174	H8	ST13	*stx2b*	cba, cea, celb, cia, cma, ehxa, espi, gad, iha, irea, iss, lpfa, senb, stx2a, stx2b, suba, terc, tia, trat	
ED1257	Human (unknown)	2018	O174	H8	ST13	*stx2b*	cba, cea, celb, cia, cma, ehxa, espi, gad, iha, irea, iss, lpfa, senb, stx2a, stx2b, suba, terc, tia, trat	
ED1261	Human (unknown)	2018	O174	H8	ST13	*stx2b*	cba, cea, cia, cma, ehxa, espi, gad, iha, irea, iss, lpfa, ompt, senb, stx2a, stx2b, suba, terc, tia, trat	173
ED1408	Animal (deer)	2018	O174	H8	ST13	*stx2b*	cba, cea, cia, cma, ehxa, espi, gad, hra, iha, irea, iss, lpfa, ompt, senb, stx2a, stx2b, terc, tia, trat	
220492-64_CA_IZSLER	Human (unknown)	2018	O174	H21	ST677	*stx2c stx2d*	gad, hra, iha, iss, lpfa, stx2a, stx2b, terc, tia, trat	
ED1365	Food (bovine meat)	2019	O174	H28	ST?	*stx1a stx2a*	cdtb, cea, celb, cia, cib, cvac, ehxa, epea, espp, gad, iha, iss, lpfa, ompt, saa, stx1a, stx1b, stx2a, stx2b, suba, terc, trat	
ED1515	Food (unknown)	2021	O174	H2	ST661	*stx1a stx2a*	ehxa, espp, gad, hra, iha, iss, lpfa, papc, saa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1506	Food (unknown)	2021	O174	H21	ST677	*stx2c stx2d*	asta, gad, hra, iha, iss, lpfa, stx2a, stx2b, terc, tia, trat	58
ED1505	Food (unknown)	2021	O174	H21	ST677	*stx2c stx2d*	asta, gad, hra, iha, iss, lpfa, stx2a, stx2b, terc, tia, trat	58
220492-19_CA_IZSLER	Human (unknown)	2022	O174	H8	ST13	*stx1c*	celb, espi, gad, iha, irea, iss, lpfa, ompt, stx1a, stx1b, suba, terc, tia	
ED1601	Human (HUS)	2022	O174	H21	ST677	*stx2c*	cea, cia, cib, epea, espi, gad, iha, iss, lpfa, ompt, stx2a, stx2b, terc, tia, trat	
ED1583	Human (unknown)	2022	O174	H2	ST661	*stx1a stx2a*	ehxa, espp, gad, hra, iha, iss, lpfa, papc, saa, stx1a, stx1b, stx2a, stx2b, terc, trat	
ED1644	Food (unknown)	2023	O174	H21	ST677	*stx2c stx2d*	ehxa, espi, espp, gad, iha, iss, lpfa, ompt, papc, saa, stx2a, stx2b, suba, terc, tia, trat	
ED1654	Food (unknown)	2023	O174	H21	ST677	*stx2c stx2d*	ehxa, espi, espp, gad, iha, iss, lpfa, papc, saa, stx2a, stx2b, suba, terc, tia, trat	